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CERTAIN PHASES OF ENZYME ACTIVITY IN SEEDS[1/]

by

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Ripening of seeds involves a synthesis of substances of a high molecular weight. During germination the situation is reversed and the storage substances are hydrolyzed. In both cases many changes involving oxidation and reduction take place, and the whole process of ripening and germinating is accompanied by enzyme activity. Without attempting to discuss the role of enzymes in such processes as respiration of seeds, to discuss the oxidation and reduction systems involved, or to present a complete list of enzymes found in seeds, it may be of interest to review here certain findings pertaining to this enzyme activity in the ripening and germinating of seeds.

The Substrates

The reserve substances accumulated in seeds are quite varied in their nature. Carbohydrates, fats, and proteins usually predominate, but in addition to these three major groups, seeds contain hemicelluloses, glucosides, phosphatides, alcoloids, and other substances.

It is customary to divide all seeds into two groups: the oily and the starchy seeds. The following table shows the constituents of the seeds of these two groups:

1/ This note is a review of the literature. It is not offered as an original contribution, except insofar as it undertakes to interpret the present significance of previous work, in a subject which has become a critical problem in watershed erosion planting.

	Fats	Carbohydrates	Proteins
Oily seeds	Percent	Percent	Percent
Sunflower	53	11	30
Poppy	41	23	19
Almond	53	19	22
Starchy seeds			
Wheat	1.8	69	12
Corn	5.8	66	10
Rice	1.3	77	7

In general, plants with oily seeds predominate in nature, comprising about 90 percent of all plants on earth. As fats contain less oxygen and have a high energy value, they form more compact reserves of nutritive substances than do the carbohydrates.

Protein compounds generally form the smaller part of a seed's reserves, rarely reaching 25 percent of its total weight. Oily seeds usually contain a larger percentage of proteins than starchy seeds. The starchy seeds of legumes, however, are very rich in proteins, lupines containing as much as 40 percent.[2] (1)

Hemicelluloses, in addition to the part they play in the structure of the cell wall, may also function as reserve material of seeds. "Schultze[3] (2), who demonstrated the presence of hemicelluloses in the cell walls of the endosperm of many seeds, also showed that hemicelluloses diminish during germination. He stated, however, that true cellulose does not constitute a reserve."

The Enzymes

Maquenne (10)[4] and his co-workers found in grain seeds an enzyme amylo-coagulase which causes precipitation of dissolved starch. It is difficult to say whether or not the amylo-coagulase is a specific enzyme. According to Maquenne, it is not identical with the starch-hydrolysing amylase.

2/ Maximov. A textbook of plant physiology. McGraw Hill, New York. 1930.

3/ Onslow. Principles of plant biochemistry, page 1. Cambridge. 1931.

4/ Italic numbers in parentheses refer to the list of Literature Cited, pages 10 and 11.

According to Oparin and Djatschkow (13), when heads of wheat cut off in the early stages or ripening with 5 to 7 cm of stem, were placed in nutrient solution, the grain showed no later increase in amylase, but a marked increase in catalase and peroxidase. Since the grains of intact plants showed increase in all three enzymes, it was concluded that amylase is manufactured in some other parts of the plant and passes into the grains, whereas the other two are synthesized in the grain.

The changes in the quantity of enzymes (catalase, peroxidase, amylase, and protease), in ripening seeds were found by Bach, Oparin and Wahner (1) to follow a very irregular and jumping course. In spite of these irregularities, the general trend of curves was the same for all four enzymes studied. At first a rise took place, then a certain maximum was reached, and lastly a more or less sharp decline.

The quantity of enzymes in ripening seeds gradually decreased and at maturity remained at a certain level. The decrease in catalase and peroxidase was rather slight, but increased during germination roughly 2 to 5 times. Amylase and protease disappeared almost entirely in the mature seeds, but with germination a 20 to 30-fold increase was observed. N. N. Ivanov (5) quotes some results obtained by one of his associates on dynamics of enzyme action in ripening squash seeds.

Days	Catalase activity	Saccarase activity	Lipase activity
	cc. $KMnO_4$	cc. $KMnO_4$	cc. $\frac{N}{10}$ KOH
22	230.0	182.5	13.35
25	278.0	161.8	11.30
29	129.5	171.5	9.45
44	131.5	75.0	4.15
80	118.5	- -	- -

It is seen that towards the maturity of the seeds, there was a decrease in activity of all these enzymes. Probably this is true for all enzymes found in the seeds.

As regards the activity of fat-hydrolyzing enzymes, Wetter (15) has observed that lipase hydrolyses the fats only when concentration

of the medium[5] is below a certain limit. As soon as this limit is reached, the enzyme begins to synthesize the fat from the product of its hydrolysis. Again the necessity of high concentration of the substrate was emphasized by Jalander (8). He used for fat synthesis

40 gr. oleic acid
100 gr. glycerol
0.5 gr. lipase (from Ricinus seeds)
5 gr. water

The products of this synthesis were mono- and tri-glycerides.

The fat-synthesizing enzyme (S. Ivanov (7) can be obtained both from the ripening and from the germinating seeds. The hydrolysis and synthesis of fats is performed by the same enzyme, the action depending entirely upon the conditions under which the substrate is formed. In diluted solutions hydrolysis occurs, whereas in concentrated media, synthesis takes place. This corresponds closely with the processes under natural conditions, wherein hydrolysis prevails during germination of seeds when water is supplied in abundance, whereas synthesis is confined to the ripening seeds. In the latter case, under strain of transpiration, the enzyme and the substrate interact in concentrated solutions of glucose and amino-acids.

S. Ivanov (7) quotes an interesting experiment in which flax seeds were taken in a partly ripened stage and divided into two portions. One portion was dried to a constant weight and immediately analysed; the other one was kept in a humid chamber through which a slow current of air was drawn. The following table shows the result:

	Oil percent	Acid No.	Sapon. No.	Iodine No.
First portion	19.59	6.7	189.2	169.12
Second portion	18.3	13.95	189.1	160.0

In other words, as soon as the supply of the materials was cut off and concentration of the medium decreased, owing to the excess of moisture and subsequent imbibition, the synthesizing action of lipase ceased, and immediately hydrolytic action started.

5/ By concentration of the medium it is understood, not the concentration of fat or products of its hydrolysis, but rather the concentration of sugars, amino-acids, etc., that are transported to the seed during the process of ripening.

Distribution of the proteolytic enzymes in the seeds has been discussed in a recent work of Blagoweschensky and Melamed (2). The proteolytic systems seem to be different in different plants, as is seen from the following table of relative activities of these enzymes, on the basis of 100 for the maximum:

Species	Cathepsin	Cathepsic polypeptidase	Di-peptidase
Phaseolus vulgaris	33	56	45
Vicia sativa	100	--	56
Soja hispida	67	100	2
Brassica rapa	22	94	10
Thlaspi arvense	33	44	11
Ricinus communis	78	78	100

This difference is also evident in respect to glucosydases. Leoncini (9) tested the influence of extracts obtained from seeds at rest and from swelling seeds of several species on hydrolysis of arbutin, salicin and phloridzin, with the following results, (crosses indicate a positive response):

Seeds	Resting			Swelling		
	Arbutine	Salicin	Phloridzin	Arbutine	Salicin	Phloridzin
Ricinus	-	-	-	+	-	-
Marshmallow	-	-	-	+	-	-
Peanut	-	-	-	+	-	-
Sage	-	-	-	+	-	-
Nasturtium	-	-	-	+	+	-
Buckwheat	+	-	-	+	-	+
Barley	+	-	+	+	-	+
Beet	+	-	+	+	-	+
Hemp	+	-	+	+	-	+
Aconite	+	-	+	+	+	+
Wheat	+	-	+	+	+	+
Lathyrus (sativus	+	+	+	+	-	-

Simultaneously with decomposition of starch, fats, and proteins, the cell walls of the endosperm also are dissolved during the process of germination. Brown and Morris[6/] claimed to have precipitated from an extract of germinating barley an enzyme which would hydrolyse the cellulose walls of the endosperm of grasses. It was thermolabile and was termed by them "cytase". Bourgelot and Herissey[6/] showed that during the germination of the Carob bean, an enzyme is produced which converts the polysaccharides of the bean into mannose and galactose. Later it was found that enzymes (seminases) capable of hydrolysing manno-galactans and galactans into their derivative mono-saccharides, occur in the seeds of alfalfa and some other plants. Paton, Nanji and Ling[5/] have given evidence of the presence of an enzyme in the ivory nut (Phytelephas) which hydrolyses the hemicelluloses of the cell wall; 200 grams of ivory nut shavings in water kept at 45°C for six days gave 45 grams of a reducing sugar; mannose was identified as a product of hydrolysis.

6/ Cited by Onslow.

An extensive literature has been written on the enzyme activity of the seeds which require an after-ripening chilling before they are able to germinate. The enzyme activity usually was studied while the seeds were stored in some moist medium at a low temperature, (in most cases 5°C). The following list gives a tabulated summary of the findings:

Author	Reference	Plant	Catalase	Peroxidase	Oxidase
Eckerson	Bot.Gaz.55 286(1919)	Crataegus	increases	increases	appears at end of period
Rose	Bot.Gaz.67 281(1919)	Tilia	increases	-	increases
Crocker & Harrington	J.AG.R.15 137(1918)	In general	parallels physiological activity	-	-
Johns	Bot.Gaz.69 127(1920)	Acer	increases	increases slightly	-
Pack	Bot.Gaz.71 32(1921)	Juniperus	doubles	-	-
Sherman	Bot.Gaz.72 1(1921)	Amaran-thus	stable	-	-
Ota	Bot.Gaz.80 288(1925)	Xantium	increases	-	-
Davis,O.H.	Bot.Gaz.84 225(1927)	Cornus	increases	-	-
Davis,W.C.	Pl.Phys.6 127(1931)	Increase in phenolase			
Flemion	Ctr.BoyceTh 5,91(1933)	Rhodotypus	increases	increases	increases

The works listed above are purely of an empirical nature, and in most of them only catalase activity was recorded, probably because of the difficulties in accurate determination of oxygenases and peroxidases. Hydrolyzing enzymes, with one exception only, have been omitted in these studies.

A few words should be said of attempts to induce enzyme activity in resting seeds. In the practical forcing of flowering plants many stimulants have been used, such as ether, cyanic acid, warm baths. The underlying idea of these stimulants lies, apparently, in promoting development of oxidizing and hydrolyzing enzymes. Recently much attention has been paid to the use of ethylene and related substances to hasten ripening of the fruits. Harvey (4) claimed that the role of ethylene is that of inducing enzyme activity and that this induction is possible only when temperature, respiration, and condition of the hydrolytic enzymes are favorable. But Englis and Zannis (3) failed to notice any increase of the enzyme activity in a starch solution subjected to the action of diastase. The same results were obtained with yeast saccharase. Action of ethylene on the enzymes is apparently not a direct one since only when ethylene acted on the living protoplasm, was an increase in the enzyme activity noted. To Nord and Franke (11), the action of minute quantities of ethylene and related substances on cell systems appeared to be an initial increased cell permeability allowing an intensified interaction between reactant and enzymes.

This question was more fully discussed in another paper by Norde and Franke (11), who investigate the possibility of finding a method by which the activity of enzymes could be increased. Ethylene $\begin{Bmatrix} CH_2^+ \\ \| \\ CH_2^- \end{Bmatrix}$ might be electrically charged on the enzyme surfaces, in this way forming an adsorbed film, and since its opposite charges are neutralized in the condition of adsorption, it does not hinder the formation of the enzyme-substrate complex, but might act as a protector of the surface upon which it is adsorbed.

Ivanov, Prokochev and Gabuinia (6) found that, under the influence of ethylene, respiration of cells is increased 300 times. Owing to an increase in oxygen, enzymetic activity is greatly increased, at the expense of the autolysis of the cells. From this a deduction can be made, that in germinating seeds under the influence of ethylene, the permeability of the cells is increased, thus promoting the reactant-enzyme complex and in this way advancing hydrolysis of stored products and increasing the oxidation-reduction potential. Under the influence of ethylene, the peroxidase content in ripening fruits, (and seeds) is greatly increased, (5), but catalase activity is somewhat decreased.

Juice from cucumbers 10 cc. aliquot	Peroxidase activity cc. $\frac{N}{10}$ $KMnO_4$	Catalase activity $\frac{N}{10}$ $KMnO_4$
Ethylene (1/1000-20 hrs.	344.8	6.15
Control	173.2	8.18

In another experiment, green tomatoes were kept in air and in 1:2000 ethylene for 10 days: Results obtained were as follows:

	Catalase activity $\frac{N}{10}$ $KMnO_4$
Control	54.59
Ethylene	18.86

This initial fall in the catalase activity with consequent increase was also observed by Stephan (14) in germinating seeds. It apparently was caused by initial increase in respiration. Later additional catalase is formed. Of course, the difference between the germinating seeds and the ethylene-treated fruits is that in the latter case no subsequent increase in catalase takes place. As a whole, the question of the influence of ethylene on the activation of the enzymes appears to be still in a controversial state. It is not altogether certain whether it is actually involved in the enzyme-substrate reaction, or concerned chiefly with changes in permeability of the protoplasm.

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